PEDIATRIC SCIENCES JOURNAL
The Official Journal of the Pediatric Department, Faculty of Medicine, Cairo University, Egypt

Original Article

Non- Disjunction of Chromosome 21 in the Young Mother at Conception

Hala Ahmed ElGindy[1], Magd Ahmed Kotb[1], Mohamed Farouk Mohamed[1], Walaa Alsharany Abuelhamd[1], Nahed Naguib Alsabagh[2], Nancy Anis[3], Aly Elkazaz[1]*

[1] Department of Pediatrics, Faculty of Medicine, Cairo University, Egypt; elgindyhala@gmail.com, magdkotb@kasrakainy.edu.eg, m.farouk76@gmail.com,walaa1@gmail.com
[2] Department of Pediatrics, Ministry of Health Hospitals, Department of Pediatrics, Ministry of Health Hospitals, Fawzy Moaz Pediatric Hospital, Alexandria**,** Egypt; nahedalasabagh@yahoo.com
[3] Department of Cardiology, School of Medicine, National University in Ireland, Galway, Ireland; nancy.anis@nuigalway.ie
***** Correspondence: alyelkazaz1@gmail.com
Received: 5/6/2022; Accepted: 18/6/2022; Published online: 1/7/2022

Abstract:

Background: Maternal age influences the type of chromosomal anomaly in Down syndrome. The older age is associated with non-disjunction while the younger age is associated with translocation of chromosome 21.
Aim of the Work: To study the cytogenetics of children with Down syndrome born to the young mother who presented to Pediatrics Genetics Unit at Cairo University Children Hospital during one year (2019), and compare with those born to an older mother.
Materials and Methods: This descriptive retrospective study analyzed records of 210 children with Down syndrome who presented to Pediatrics Genetics Unit at Cairo University Children Hospital during 2019.
Results: Among the total 210 studied children with Down syndrome, 21(10%) were born to young mothers aged 21 years or less at conception. Seven (33.34%) were males and 14 (66.6%) were females. Twenty (95.2%) were first born to a young mother, only 1 was a second born. Karyotyping proved that 6 (28.6%) had Robertsonian translocation of trisomy 21, and 15 (75.4%) had non-disjunction. Only 1 (4.76%) of children born to younger mothers had associated cardiac anomalies, while among those born to the older mother 40 (21%) had associated anomalies, of them were cardiac 36 (19.04%), hydrocephalus in one (0.5%), and ambiguous genitalia in 3 (1.58%) (p=0.072). First born trisomy 21 were 109 (51.9%) among the whole studied cohort, with a mean maternal age +/- SD of 30.2 years +/- 7.35 years, their karyotyping was non-disjunction, Robertsonian translocation and mosaic in 100, 5 and 3 respectively. Cardiac anomalies were encountered among 18 (16.5%).
Conclusion: Children born with trisomy 21 to mothers younger than 21 at conception are mostly due to non-disjunction. First born children with trisomy 21 comprised half the studied cohort of trisomy 21. Further studies to define preventable causes of non-disjunction in the young mother, are needed to reduce the incidence of Down syndrome.

Level of Evidence of Study: IIB. (*1*)
Keywords: Down syndrome; non-disjunction; young mother; trisomy 21.
Abbreviations: ASD: Atrial septal defect; CAV: complete atrioventricular canal; VSD: ventricular septal defect.

Introduction

It has been long noted that maternal and paternal age advancement is associated with increased risk of non-disjunction trisomy 21 off-springs (*2*, *3*). Trisomy 21 or Down syndrome is a common numerical chromosomal anomaly. Survival of afflicted subjects is ever increasing from 10 years of those with Down syndrome in the 1960s to almost 47 years in 2007. Hence, the increased prevalence of Down Syndrome, and the growing need to address their medical and

social needs for smooth integration in society (*4*, *5*). Parents age advancement is noted in highly educated societies compared to other less educated societies (*6–9*). With the noted advancement of parental age and the increased risk of non-disjunction, it is less expected to come across young mothers. As only one trisomy is expected for every 1,250-2000 in a woman who conceives at age 25, compared to 1 in a hundred who conceives at 40 years (*10*). Cytogenetics and karyotyping allows delineation of the chromosomal anomaly underlying the trisomy 21 among children with Down Syndrome. The trisomy 21 in a baby born to a young mother is more likely attributed to translocation carriage or mosaicism, than in the older mother (*11*, *12*). The aim of this work is study the cytogenetics of children with Down syndrome born to the young mother who presented to Pediatrics Genetics Unit at Cairo University Children Hospital during one year (2019), and compare with those born to an older mother.

Subjects and Methods

This retrospective cohort study involved data of 210 children with Down Syndrome following up at the Pediatrics Genetics Unit, Children Hospital, Cairo University Hospitals during one year (2019). The study was approved by the Ethical Committee of Cairo University Pediatric Department and Higher Education Research Committee of Faculty of Medicine, Cairo University, Egypt. An informed consent was obtained from the patients' parents.

All data of children with Down Syndrome with mothers aged 21 and 9 months or less years at birth were included in the study. Data of those with higher maternal age was used for comparison.

Methods

- Data Collection:

Of all relevant clinical features, maternal age, family history, parental consanguinity, investigations and associations if any.

- Karyotyping

1. **Culture and Harvesting:** A total 0.5 ml of blood sample was culture in 5ml RPMI 1640 (gibco ready to used media) in a 15ml screw cap culture vials under aseptic precautions. Incubation was done in CO2 incubator at 37°C, 5% CO2 inject and 84% humidity (Incubator values set) for 70 hours.
2. **Banding (Trypsin Digestion)** in Giemsa solution for 5 to 7 minutes. The slides were mounted with coverslip using distyrene, a plasticizer, and xylene (DPX) solution.
3. **Metaphase Analysis:** 20-25 metaphases were examined and 3- 5 cells were photographed and karyotyped. In case of mosaicism, 50 to 100 metaphases were scored. Karyotype description was done according to the international nomenclature guidelines (ISCN) (*13*)**.**

Statistical Analysis

All data was tabulated and analyzed using Statistical Package for Social Sciences (SPSS) (version 20). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) and Fisher exact was used to calculate difference between qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation) and/or median and range. Mann Whitney test was used to calculate difference between quantitative variables in the two groups for non-parametric variables. All statistical comparisons were two tailed with significance level of p value ≤ 0.05.

Results

The median of age of referral of Down syndrome are 4 months with range between 1 to 150 months, while the median of their mothers was 34 years with range between 17 to 47 years. The studied group comprised 210 children with Down, of them 107 (51.9%) were males, mostly 1st sibling (51.4%), mostly from Urban areas (57.6%), 29.5% with positive parental consanguinity, and only 8.1% with history of same condition in the family as presented in table 1. Among the total 210 studied children with Down syndrome, 21(10%) were born to young mothers aged 21 years or less at conception. Table 1 shows the characteristics of trisomy 21 according to maternal

age at conception of the mothers younger than 21 at conception. Seven (33.34%) were males and 14 (66.6%) were females. Twenty (95.2%) were first born to the young mother, only 1 was a second born. Karyotyping proved that 6 (28.6%) had Robertsonian translocation of trisomy 21, and 15 (75.4%) had non-disjunction.

Table 1. Demographic characteristics of Down syndrome (N=210).

		Median	Range
Age at referral		4.0	1-150
Mother Age		34	17-47
Variable		N	%
Sex	F	103	48.1%
	M	107	51.9%
Sibling order	1st	108	51.4%
	2nd	43	20.4%
	3rd	43	20.4%
	4th	14	6.6%
	5th	2	0.9%
Residence	Rural	89	42.4%
	Urban	121	57.6%
Positive consanguinity		62	29.5%
History of same condition in the family		17	8.1%

Table 2. Trisomy 21 characteristics according to maternal age up to 21 years old at conception among 21 studied children.

Gender		Order among sibs		Positive Parental Consanguinity	Associated Congenital Anomalies		Karyotype	
Females	Males				Number	Type		
Maternal age at conception: up to 16 years (total 2 children)								
2	0	First	2	0	0		Non-disjunction	
Maternal age at conception: 18 years (total 1 children)								
1	0	First	1	1	1	VSD	Non-disjunction	
Maternal age at conception: > 18 years up to 19 (total 2 children)								
2	0	First	2	0	0		Non-disjunction	
Maternal age at conception: >19 years up to 20 (total 5 children)								
0	5	First	5	3	0		Non-disjunction	2
							R. Translocation	3
Maternal age at conception: > 20 years up to 21 (total 11 children)								
2	9	First	10	6	0		Non-disjunction	2
		Second	1				R. Translocation*	3

*There was family history of Down syndrome in the family. R. Translocation: Robertsonian translocation; VSD: ventricular septal defect.

Table 2 shows the characteristics of children with trisomy 21 born to mothers older than 21 at conception. There was no difference among those born to the younger mother and the older mother as regards the dysmorphic features hypertelorism, multiple dysmorphic features, broad nasal bridge, prominent occiput, large tongue-silky hair, low set ear, protruded tongue, silky hair-large tongue, depressed nasal bridge, high arched palate and upward slanting eye. Only 1 (4.76%) of children born to younger mothers had associated cardiac anomalies, while among those born to the older mother 40 (21%) had associated anomalies, of them were cardiac 36 (19.04%), hydrocephalus in one (0.5%), and ambiguous genitalia in 3 (1.58%) (p=0.072). First born trisomy 21 were 109 (51.9%) among the whole studied cohort, with a mean maternal age +/- SD of 30.2 years +/- 7.35 years, their karyotyping was non-disjunction, Robertsonian translocation and mosaic in 100, 5 and 3 respectively. Cardiac anomalies were encountered among 18 (16.5%).

Table 3. Trisomy 21 characteristics according to maternal age above 21 year old at conception among 189 studied children.

Gender		Order among sibs		Positive Parental Consanguinity	Associated Congenital Anomalies		Karyotype	
Females	Males				Number	Type		
Maternal age at conception: above 21 up to 25 years (total 24 children)								
14	11	First	22	4	1	VSD	Non-disjunction	22
		Second	2		2	CAV	Mosaic	2
Maternal age at conception: > 25 years up to 29 (total 29 children)								
17	12	First	11	6	1	hydrocephalus	Non-disjunction	28
		Second	17			Ambiguous genitalia		
Maternal age at conception: > 29 years up to 34 (total 53 children)								
19	34	First	16	20	7	VSD	Non-disjunction	54
		Second	13		7	CAV		
		Third	23				Mosaic	1
		Fourth	1		1	ASD		
Maternal age at conception: > 34 years up to 39 (total 68 children)								
34	34	First	28	1	5	VSD	Non-disjunction	66
		Second	12					
		Third	18		10	CAV	Mosaic	2
		Fourth	9					
		Fifth	1					
Maternal age at conception: > 39 years up to 45 (total 15 children)								
13	2	First	7	2	1	VSD	Non-disjunction	
		Second	2					
		Third	1		2	CAV		
		Fourth	4					
		Fifth	1		2	Ambiguous genitalia		
Maternal age at conception: 56 (total 1 children)								
1	0	Third	1	0	1	Mitral Regurge	Non-disjunction	

ASD: Atrial septal defect; CAV: complete atrioventricular canal; R. Translocation: Robertsonian translocation; VSD: ventricular septal defect

Discussion

Ten percent of our studied children with trisomy 21 were born to mother who were younger than 21 at conception. Of them 75% were attributed to non-disjunction. Maternal age advancement was always noted to be associated with trisomy 21(*14*). It was attributed to a multifactorial trait that results in failure of chromosomes to segregate during meiosis. Yet, non-age related non-disjunction was reported in those with a single pericentromeric exchange in first meiotic division (*15*). We did not study the maternal failure of separation of the homologous chromosomes in first meiotic division and did not study maternal failure of sister chromatids to separate during meiosis II, stratified by maternal age. We did not study the mitotic failure of sister chromatids to separate as well. We did not study the epigenetic and environmental factors that might be responsible for this non-disjunction in the offspring of the younger mother (*16*).

The phenotype of those born to the younger mother and the older mother was not different. There was no peculiarity of dysmorphic features in either group. Yet, there was more associated anomalies in the trisomy 21 born to the older mother, that did not mount to statistical significance, with a total of 37 (17.6%) children having cardiac anomalies. The burden of trisomy

21 is not limited to the family and society only, the associated anomalies pauses other loads on other departments as cardiology and cardiosurgery, etc.

The first born child with trisomy 21 was encountered among more than half our studied cohort. Their mothers had a higher mean age at conception of 30 years +/- 7 years. The trisomy 21 is as common in first born to the older mother as in the later pregnancies of the older mother who had her earlier off springs in a younger age. It seems that advancement of age is the crucial factor that paves the way for environmental factors and epigenetic factors to interplay (*16*).

The lack of phenotype and the majority of non-disjunction, limit prenatal diagnosis to translocation carriers, and highlight the importance of Down syndrome diagnosis during early pregnancy. Sensitivity and specificity of tests for diagnosis of Down syndrome as detection of fetal nuchal translucency, the pregnancy associated plasma protein-A, free human chorionic gonadotropin in maternal blood, cell-free fetal DNA second-generation sequencing technology in maternal blood are 17.85% and 85.71%, 62.50% and 87.35%, 58.93% and 86.62% (*17*) and 97% and 99% (*18*) respectively. The later relies on sequencing of fetal cells and fetal stem cells that traffic into the maternal circulation during pregnancy.

The incidence of Down syndrome in Egypt is 1:555 and 1:770 life births(*19*). We recommend establishment of a registry of cases with trisomy 21 all over Egypt. Studies of cost-effectiveness of the highly sensitive non-invasive sequencing tests are needed, yet with the advancement of technology and its availability, we support its routine use during pregnancy of the advanced in age parents, and if possible among all pregnancies even among the younger mother. Prenatal screening for Down syndrome is vital to reduce the disease burden of Down syndrome, and reduce the burden of mental sub-normality, associated anomalies, etc.

A limitation of this study is that we did not study influence of advancing paternal age on the type of trisomy 21, as the paternal contribution to trisomy 21 in the offspring is 50% when maternal age is above 35 (*20*). Again we did not study the potential causes of the non-disjunction among our studied cohort. We did not study inactivation of topoisomerase II, separase or condensin (*21*) that are potential causes for non-disjunction as they were beyond the scope of the study.

Conclusion

Children born with trisomy 21 to mothers younger than 21 at conception are mostly due to non-disjunction. First born children with trisomy 21 comprised half the studied cohort of trisomy 21. Further studies to define preventable causes of non-disjunction in the young mother, are needed to reduce the incidence of Down syndrome. Multidisciplinary medical, counseling, family planning and social support for young parents of the Down syndrome are vital.

Author Contributions

All authors searched medical literature, databases, conceptualized, conducted the case review and reviewed the final manuscript. All authors have read and agreed to the published version of the manuscript.

FUNDING

Authors declare there was no extramural funding provided for this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest in connection with the reported study. Authors declare veracity of information.

References

1. S. Tenny, M. Varacallo, *Evidence Based Medicine.* (StatPearls Publishing; Treasure Island (FL), 2020; https://www.ncbi.nlm.nih.gov/books/NBK470182/).
2. T. A. Bruckner, P. Singh, N. Lelong, B. Khoshnood, Down syndrome among primiparae at older maternal age: A test of the relaxed filter hypothesis. *Birth Defects Research*. **111**, 1611–1617 (2019).

3. Hafez, M., M. El-Tahan, M. Zedan, and M. Eisa, Demographic Trends of Down's Syndrome in Egypt. **56**, 703–12 (1984).
4. A. P. Presson, G. Partyka, K. M. Jensen, O. J. Devine, S. A. Rasmussen, L. L. McCabe, E. R. B. McCabe, Current Estimate of Down Syndrome Population Prevalence in the United States. *The Journal of Pediatrics*. **163**, 1163–1168 (2013).
5. Special Olympics, Down Syndrome, (available at https://www.specialolympics.org/tag/down-syndrome).
6. J. Knodel, N. Havanon, W. Sittitrai, Family Size and the Education of Children in the Context of Rapid Fertility Decline. *Population and Development Review*. **16**, 31 (1990).
7. A. B. Babar, Education is the best contraceptive (2016), doi:10.13140/RG.2.2.11763.73765.
8. M. Janecka, F. Rijsdijk, D. Rai, A. Modabbernia, A. Reichenberg, Advantageous developmental outcomes of advancing paternal age. *Transl Psychiatry*. **7**, e1156 (2017).
9. S. Cantalini, R. Guetto, N. Panichella, Parental age at childbirth and children's educational outcomes: evidence from upper-secondary schools in Italy. *Genus*. **76**, 8 (2020).
10. C. T. Mai, J. E. Kucik, J. Isenburg, M. L. Feldkamp, L. K. Marengo, E. M. Bugenske, P. G. Thorpe, J. M. Jackson, A. Correa, R. Rickard, C. J. Alverson, R. S. Kirby, for the National Birth Defects Prevention Network, Selected birth defects data from population-based birth defects surveillance programs in the United States, 2006 to 2010: Featuring trisomy conditions: U.S. Trisomy Conditions: 2006-2010. *Birth Defects Research Part A: Clinical and Molecular Teratology*. **97**, 709–725 (2013).
11. E. B. Hook, Parental age and unbalanced Robertsonian translocations associated with Down syndrome and Patau syndrome: comparison with maternal and paternal age effects for 47, + 21 and 47, + 13. *Ann Human Genet*. **48**, 313–325 (1984).
12. F. D. Bricarelli, M. Pierluigi, M. Landucci, A. Arslanian, D. A. Coviello, M. A. Ferro, P. Strigini, Parental age and the origin of trisomy 21: A study of 302 families. *Hum Genet*. **82**, 20–26 (1989).
13. M. Stevens-Kroef, A. Simons, K. Rack, R. J. Hastings, in *Cancer Cytogenetics*, T. S. K. Wan, Ed. (Springer New York, New York, NY, 2017; http://link.springer.com/10.1007/978-1-4939-6703-2_24), vol. 1541 of *Methods in Molecular Biology*, pp. 303–309.
14. E. G. Allen, S. B. Freeman, C. Druschel, C. A. Hobbs, L. A. O'Leary, P. A. Romitti, M. H. Royle, C. P. Torfs, S. L. Sherman, Maternal age and risk for trisomy 21 assessed by the origin of chromosome nondisjunction: a report from the Atlanta and National Down Syndrome Projects. *Hum Genet*. **125**, 41–52 (2009).
15. T. R. Oliver, E. Feingold, K. Yu, V. Cheung, S. Tinker, M. Yadav-Shah, N. Masse, S. L. Sherman, New Insights into Human Nondisjunction of Chromosome 21 in Oocytes. *PLoS Genet*. **4**, e1000033 (2008).
16. F. Coppedè, Risk factors for Down syndrome. *Arch Toxicol*. **90**, 2917–2929 (2016).
17. J. Durković, M. Ubavić, M. Durković, T. Kis, Prenatal Screening Markers for Down Syndrome: Sensitivity, Specificity, Positive and Negative Expected Value Method. *J Med Biochem*. **37**, 62–66 (2018).
18. M. Rosner, T. Kolbe, M. Hengstschläger, Fetomaternal microchimerism and genetic diagnosis: On the origins of fetal cells and cell-free fetal DNA in the pregnant woman. *Mutation Research/Reviews in Mutation Research*. **788**, 108399 (2021).
19. H. S. Abou-Youssef, M. M. Kamal, D. A. Mehaney, Triple test screening for Down syndrome: an Egyptian-tailored study. *PLoS One*. **9**, e110370 (2014).
20. H. Fisch, G. Hyun, R. Golden, T. W. Hensle, C. A. Olsson, G. L. Liberson, The Influence of Paternal Age on Down Syndrome. *Journal of Urology*. **169**, 2275–2278 (2003).
21. Samantha F. Gottlieb; Connor Tupper; Connor C. Kerndt; David H. Tegay., in *Genetics, StatPearls [Internet]*. (Treasure Island (FL): StatPearls Publishing;, 2022; https://www.ncbi.nlm.nih.gov/books/NBK482240/).